

ELISA inhibition method in detection of mite and chironomid antigens in environmental samples of dust, soil and air

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To determine the amount of chironomids and mite antigens in the indoor and outdoor environment, we investigated samples from air, soil and house dust in the area around Lake Kojima, located in the western part of Japan. Three species of chironomid (*Tokunagayusurika akamusi*, *Chironomus yoshimatsui* and *Chironomus plumosus*) and a species of mite, *Dermatophagoides farinae* antigens were studied. The antigens were detected and quantified by inhibition ELISA. In the outdoor environment, *C. yoshimatsui* and *T. akamusi* antigens were more abundant than mite antigen, and seasonal fluctuations were observed. In the indoor environment, mite was the predominant antigen. *D. farinae* antigen was detected in almost all dust samples, with a slight reduction in winter compared to summer.

Key words: air sampling; allergen; chironomid antigens; ELISA; environmental antigens; house dust; mite antigens; soil sampling.

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Many arthropods have been studied as possible or recognized agents causing allergic diseases, such as bronchial asthma, rhinitis and hypersensitivity pneumonitis. Mite has been known as one of the most prevalent allergens (7, 14, 20). Chironomid midge is recently incriminated as a source of allergen in Africa and Europe (6, 8). In Japan, some authors already consider chironomids as an important cause of such diseases (18). Around Lake Kojima, in Okayama prefecture, Japan, an increasing number of chironomids have been observed in the last 6 years. Matsuoka et al. (17) demonstrated a high titer of specific IgE antibody to chironomids in the sera of asthmatic children living around the lake. To prove the existence of chironomid and mite antigens in the environment, samples from air, soil and house dust were studied by means of the ELISA inhibition method.

MATERIAL AND METHODS

Dermatophagoides farinae were cultured according to the method of Sasa et al. (24) and processed by the method of Abe et al. (1). *Tokunagayusurika akamusi* and *Chironomus yoshimatsui* were collected around Lake Kojima and *Chironomus plumosus* around Lake Biwa, situated in the central part of Japan. The adult chironomids and mites were homogenized with 0.1 M Tris-hydrochloric acid buffer (pH 7.6), stirred for 20 h at 4°C, and centrifuged at 100,000 × *g* for 1 h (9, 17). Each supernatant was used as a crude antigen. Protein concentration was measured by the method of Lowry et al. (15).

Sera of immunized Japanese white rabbits (from Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were used as the first antibody. Each rabbit was injected subcutaneously

with 15 mg of a crude antigen with Freund complete adjuvant (FCA). At 2-week intervals, injections were repeated three times with 10 mg of the same antigen with FCA. Seven days after the last injection, sera were taken from the rabbits, and stored at -20°C until use (9).

A high-volume air sampler, HVS-1000 (Shibata Kagaku Co. Ltd., Tokyo, Japan), was used for air sampling (13). This sampler was set with two glass-fiber filters, where the upper filter retained all particles larger than $10\text{ }\mu\text{m}$ in diameter, and the lower filter caught particles smaller than $10\text{ }\mu\text{m}$. We examined the smaller particles retained on the lower filter, as these could enter the lung and cause bronchial asthma (3). The air sampler was placed at the lake side and activated each month from November 1987 to February 1989 for 10 consecutive days with a constant air volume of 1500 l/min . The antigens on the filter were extracted by the descending elution method (2, 28) with 500 ml of 0.02 M phosphate-buffered saline (PBS), pH 7.2. The eluate was concentrated to 10 ml by ultrafiltration with 5000 MW cutoff filter membrane (Amicon Corporation, Danvers, Ireland). It was then lyophilised and reconstituted in 3 ml PBS.

Soil was collected each month from October 1986 to December 1987 at three different places around Lake Kojima. The superficial layer (10 mm in depth) was removed and sieved for 30 min through a mesh #9 (9 mesh screens/inch). Five grams of the sieved clay were mixed with 10 ml of PBS-Tween 20 (0.05% Tween 20 in PBS), stirred for 30 min and centrifuged at $1500 \times g$ for 10 min . The supernatant was collected and stored at -20°C until testing.

Dust was collected from the electric cleaners of 22 households in August and of 16 households in December, 1985. All houses sampled were situated around Lake Kojima. Eight of the houses were sampled in both August and December. One gram of fine dust (sieved through mesh #9 and retained on mesh #200) mixed with 10 ml PBS was stirred for 30 min and centrifuged at $1500 \times g$ for 10 min (10). The supernatant was kept in vials and stored at -20°C until testing.

The antigen was assayed by the ELISA (enzyme-linked immunosorbent assay) inhibition

method. The arthropod antigens determined were: *T. akamusi* adult (TAA), *C. yoshimatsui* adult (CYA), *C. plumosus* adult (CPA) and *D. farinae* (DF). Firstly, a 96 flat microwell polystyrene plate (Nunc, Roskilde, Denmark) was coated with $100\text{ }\mu\text{l}$ of the crude antigen diluted to $10\text{ }\mu\text{g protein/ml}$ with 0.05 M carbonate buffer, pH 9.6. The plate was sealed with wide cellophane tape and stored overnight at 4°C . On the next day, $100\text{ }\mu\text{l}$ of each sample were added to $100\text{ }\mu\text{l}$ of the immunized rabbit serum diluted 10,000-fold in 1% BSA/PBS-Tween 20 (1% bovine serum albumin in PBS with 0.05% Tween 20) and incubated for 30 min at 37°C with shaking. The plate was washed 3 times with PBS-Tween 20, and $75\text{ }\mu\text{l}$ of the sample-rabbit serum (prepared above) were placed in each well in duplicate. After 1 h incubation at 37°C , the plate was washed 3 times with PBS-Tween 20. As the second antibody, we used $100\text{ }\mu\text{l}$ of a peroxidase-conjugated goat anti-rabbit IgG (MBL, Nagoya, Japan) diluted 4000-fold in 1% BSA/PBS-Tween 20. It was incubated again for 1 h and washed three times with PBS-Tween 20. The substrate used was $100\text{ }\mu\text{l}$ of 0.3% ABTS (2,2'-azinobis (3-ethylbenz-thiazoline sulfonic acid) (Sigma Chemical Co., USA) in 0.05 M citrate-phosphate buffer (pH 5.0) with 0.001% H_2O_2 . Thirty minutes after the substrate was placed, $50\text{ }\mu\text{l}$ of 1.25% sodium fluoride was added to stop the enzyme reaction, and absorbance was measured with a spectrophotometer (Sanko Myreader, Tokyo) at OD 410 nm. A standard curve for each antigen was made for each experiment with antigen concentrations of 100, 10, 1, 0.1, 0.01, 0.001, 0.0001 and $0\text{ }\mu\text{g protein/ml}$. The antigen content in each sample was calculated mathematically according to the standard curve.

All statistical analysis was done with the *t*-test.

RESULTS

To verify the possibility of cross-reaction among the antigens, a test was performed. The result showed a weak but significant reaction between CPA antigen with anti-CYA rabbit serum and vice-versa. TAA and DF showed no cross-reac-

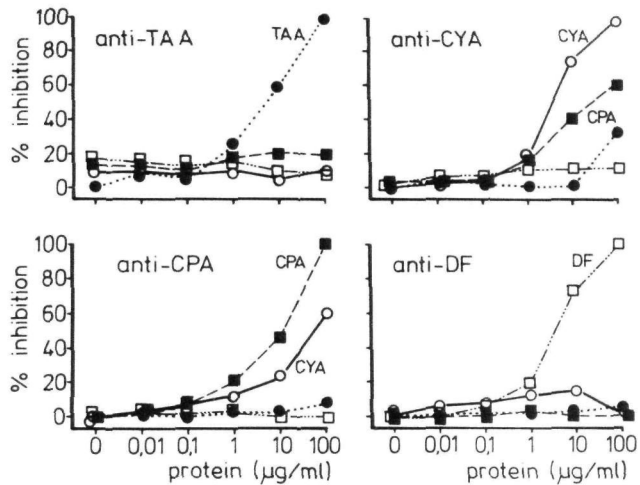


Fig. 1. Cross-reaction test among the four antigens to each respective anti-sera. ●...● TAA; ○-○ CYA; ■- ■ CPA; □-...-□ DF. A partial cross-reaction of CYA antigen to anti-CPA and also the reverse is observed. TAA and DF antigens did not show cross-reactivity with other antigens.

tion with any of the anti-sera studied (Fig. 1). Although not shown, cross-reactivity of DF with DP (*Dermatophagoides pteronyssinus*) antigen was also studied. Other authors have demonstrated, by different means, an important cross-reaction between DF and DP antigens (20, 22). The result obtained by our method supported this assertion. Thus, antigens of mite species other than DF can be implicated.

TAA, CYA, CPA and DF antigens in the air, soil and house dust samples were determined by ELISA inhibition. In this method, the detection limit of the antigen concentration was minimum 0.1 μl/ml (Fig. 2). In the air samples all four antigens showed the highest peak from

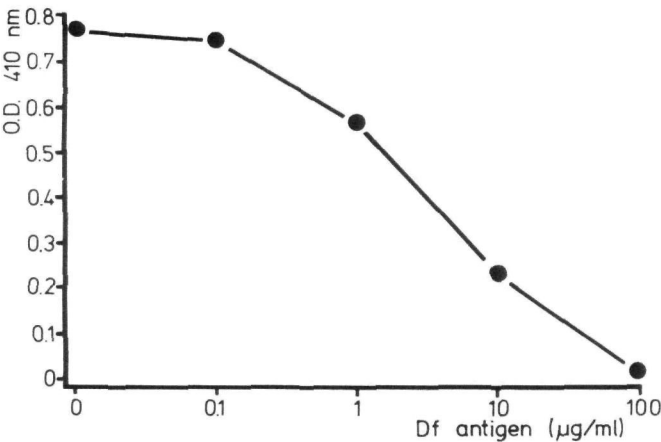


Fig. 2. Control curve of the standards of DF antigen by ELISA inhibition method.

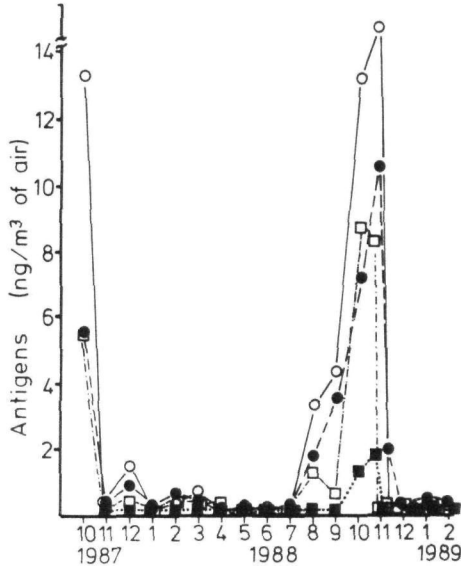


Fig. 3. Airborne antigens in the air near Lake Kojima. The air was sampled every month for 10 days from October 1987 to February 1989 with a constant volume of 1500 l/min. ○-○ CYA (*Chironomus yoshimatsui* adult); ●- ● TAA (*Tokunagayusurika akamusi* adult); ■- ■ CPA (*Chironomus plumosus* adult); □-...□ DF (*Dermatophagoides farinae*).

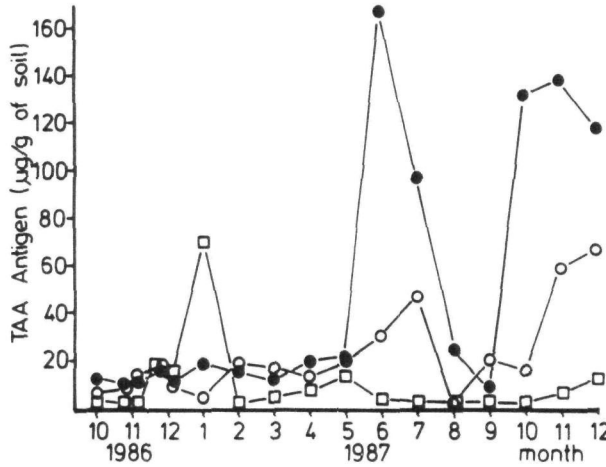
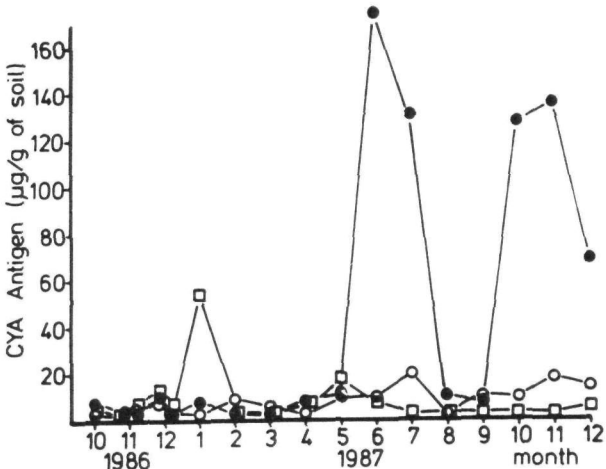


Fig. 4. TAA and CYA antigen in the soil of 3 different places: ○-○ place 1; ●-● place 2; □-□ place 3. Soil was sampled once a month from October 1986 to December 1987.

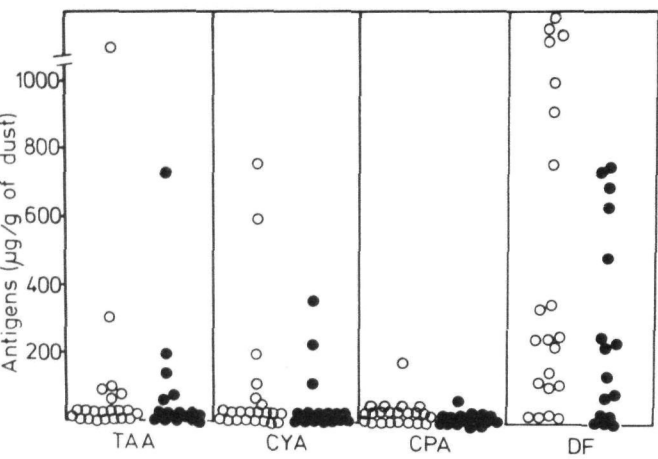


Fig. 5. Antigens detected from the dust of 22 houses in summer (○) and 16 houses in winter (●).

September to the end of November (Fig. 3). CYA was the most abundant antigen in the air, with a value of more than 14 ng/m^3 in November. TAA antigen was also high, with a maximum of 11 ng/m^3 . DF was third in abundance, with a maximum of 8.4 ng/m^3 . CPA antigen was no more than 1.8 ng/m^3 , indicating that this aeroantigen is not of great importance around Lake Kojima. From April to July, all four antigens were almost undetectable by our method.

In the soil samples, TAA and CYA antigens showed great variations according to place and season (Fig. 4). TAA and CYA exhibited a similar pattern, with a high peak in June, followed by another in November. DF antigen was detectable in only one of the three places, with a peak from June to July and another from October to November, but its maximum value was 46 µg/g soil. In contrast, the maximum value of CYA and TAA antigens in the soil was 176 and 166 µg/g , respectively. CPA antigen was less than 2.0 µg/g soil in all three places (data not shown).

To quantify the antigens in the indoor environment, we examined the dust from houses around Lake Kojima. Sampling was performed in summer and winter seasons. DF antigen was the most abundant and varied widely in amount from house to house, but in the dust from some houses there was a surprisingly high

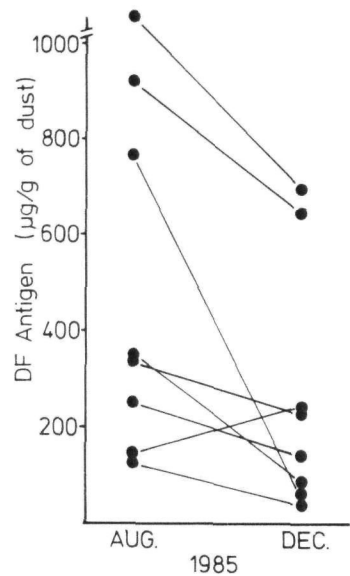


Fig. 6. DF antigen from the dust of 8 houses sampled in August and December 1985.

amount of DF antigen with values as high as 1000 µg/g dust. Since the maximum concentration of the standard used in this study was 100 µg/ml , antigen values of more than 1000 µg/g dust could not be exactly estimated. However, we observed that some dust samples showed a higher inhibitory effect for DF than the control 100 µg/ml , indicating that more than 1000 µg of DF antigen/g dust must be present in these samples. Also in winter, a large amount of DF antigen was detected. The mean value of DF antigen collected from 22 houses in summer and from 16 houses in winter did not show any significant difference between the two seasons (Fig. 5). Analysis with t-test showed no statistical difference in the antigen level of 8 houses sampled both in summer and winter (Fig. 6).

The results demonstrated that chironomid and mite antigens are abundant in our environment. According to our data, two seasons were important in regard to antigens and environment. From the end of autumn to the beginning of winter antigens were increased in quantity in all three microenvironments. This parallelism was not observed in the period spring to summer. In this season, there were high amounts of these antigens in the soil and house dust, but in the air sample the antigen measurement was low.

Independent of season, the low level of CPA antigen in the three microenvironments was

anticipated, since this midge is not common in the area under study.

DISCUSSION

Not only house dust mite allergies, but hypersensitivity to chironomids has been recognized in North Africa and West Germany (6, 9, 11). Also in Japan, allergologists have incriminated these insects as responsible for allergic diseases (13, 18). How patients become sensitized to these allergens is as yet not known. Previous studies have demonstrated mite antigens in the air of indoor environment. The dead bodies and fecal particles of mite are considered an important source of allergens, and it is usual for these particles to become airborne after disturbance in rooms (21, 30). Thus, the respiratory tract is supposed the principal site of sensitization in these patients. The present study analysed allergens in air, soil and house dust, all of which can come in direct contact with the respiratory tract.

In this study we emphasized the chironomid as an important antigen in the outdoor environment and concluded that chironomid antigen was quantitatively the most prevalent in the outdoor environment. A wide number of chironomid species are described in Japan (25). Their distribution depends on such factors as altitude, season (12) and year, but the most important factor seems to be water condition, this being closely related to chironomid proliferation (26). We studied particularly the *T. akamusi*, and *C. yoshimatsui*. In the Okayama prefecture, in areas other than around Lake Kojima, *C. yoshimatsui* was one of the predominant species encountered, especially in small streams in the urban area (29). However, in the area around Lake Kojima, *T. akamusi* predominate, especially in November (27). The presence of chironomid antigens in the air has been reported, but, as yet, there is no study of these antigens in the soil or house dust. In this present study we have demonstrated that antigens were also detected in the soil, with large seasonal and local variations. Kino et al. (13) showed seasonal fluctuations of atmospheric chironomid antigens in Kyoto, with two peaks, one in

spring and another in autumn. We demonstrated high amounts of TAA and CYA antigens in both air and soil. Despite the increased amount of these antigens in the soil in summer and autumn, the air samples showed a peak only in autumn. This might be because the antigens in the air are more easily affected by natural phenomena, especially rain. We consider that the pluviometric index during air sampling (data provided by Okayama Meteorological Observations Center) confirms this, because a higher pluviometric index was coincident with a remarkable decrease in airborne antigens. The antigen content of *C. plumosus*, a species of midges commonly encountered around Lake Biwa, but not in Okayama, was very low in both environments. Moreover, in contrast to the outdoor environment, TAA and CYA antigens in the indoor environment were inconspicuous.

However, mite antigens have a relevant importance when the sample is from the indoor environment. Although mite antigen was detected in the air and soil, this was less significant compared to the chironomid antigens. House dust mite has long been incriminated as one of the most important sources of indoor allergens (30, 31). In the United States, Lind et al. (14) reported that 95% of house dust samples contained mite allergens. Our results support that, also in Japan, mite antigen can be detected in almost all house dust samples.

The environmental temperature and relative humidity are the limiting factors in controlling mite proliferation (5, 16, 24), and it is reported that the greatest proliferation of mites occurs mainly in spring/summer, followed by a marked reduction in winter (4, 19). Despite this decrease, some authors noted that the amount of antigen in the indoor environment can remain high for several months after the mites have decreased in number (22, 23). In this study, we did not count the number of mites in each sample, and thus cannot confirm this observation. However, our result is not inconsistent with that of these authors, because a high amount of mite antigen was extracted from house dust even in winter. The elevated quantity of mite antigen throughout the year may

explain why some patients exhibit perennial allergic symptoms to house dust.

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